# *Microbial Recolonization of a Fumigated Nursery Soil*

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***Abstract.*** Soil fumigation with methyl bromide resulted in the dominance among the fungi of *Trichoderma* species and *Penicillium piscarium* in both the soil and rhizosphere. The inoculum source was a pine needle mulch applied following fumigation. Application of a microbiologically different mulch resulted in a different fungal population which was still detectable after 4 months. The fungal population in the subsurface soil remained at a very low level throughout the study period of 7 months. The bacteria responded immediately and strongly to fumigation. Numbers in the subsurface soil subjected to high levels of methyl bromide exceeded the numbers in the controls 10 to 20 times immediately after removal of the fumigation covers. Their populations fell sharply but remained above the control populations throughout the study. The fungal species isolated from the rhizosphere were the same as those isolated from the soil, but 7 of the 19 species isolated from surface-sterilized roots were not recorded from either the soil or rhizosphere. Larger numbers of fungi in genera known to include plant pathogens were isolated from the surface-sterilized root tips of seedlings grown in nonfumigated soil than from fumigated soil. Clamp-bearing basidiomycetes were isolated from roots from all treatments. Histological and morphological examinations of roots indicated that fumigation delayed mycorrhiza formation for several weeks and altered the fungal symbionts involved.

***Additional key words.*** *Trichoderma*, mycorrhizae, *Pinus taeda*, rhizosphere, *Penicillium piscarium.*

NUMEROUS studies cited by Vaartaja (1964) have shown a stimulation of tree seedling growth in forest nurseries following fumigation. The stimulation has variously been attributed to increased nutrient availability (Aldrich and Martin 1952, Smith 1963) and to changes in the microbial population in the ecosystem (Martin 1950, Kreutzer 1965, Ingestad and Nilsson 1964).

Nonselective toxic chemicals used to control weeds and pathogens will temporarily alter the soil and rhizosphere microflora (Warcup 1957). The rhizosphere microflora may affect plant growth through changes in competition for nutrients, mineralization or chelation of nutrients, elimination of organisms that produce phytotoxic substances, or inhibition of weak parasites. Wilhelm (1965) has demonstrated that elimination of *Pythium ultimum*, a pathogenic organism, by fumigation resulted in increased growth of several agricultural crops. The promotion of *Trichoderma* or other antagonistic microorganisms may also play an important role. Work with a variety of plants has demonstrated that common soil fungi can often exert stimulatory or

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inhibitory influences on the growth of shoots and roots (Domsch 1965, Rovira 1965). It is not unlikely then that radical changes of the rhizosphere microflora may eliminate fungal species capable of depressing plant growth or encourage species capable of stimulating growth.

Another microbial aspect is the possible effects of fumigation on the mycorrhizal symbionts. Mycorrhizae have been reported to be important in nutrient uptake (Harley 1959) and in protection of roots from soil-borne pathogens (Marx and Davey 1967). The retardation of mycorrhiza formation after use of methyl bromide has been frequently reported (Hacskaylo and Palmer 1957, Wright 1957, Laiho and Mikola 1964), but in most cases the growth of seedlings in fumigated soil was superior to that in nonfumigated soil. Thus it would appear that either mycorrhizae are not always essential in fumigated soils (e.g., for disease protection) or the soils are readily recolonized by highly efficient mycobionts.

Since fumigation of nursery soils is a regular cultural practice, we established an experiment to: study the growth and nutrient status of loblolly pine seedlings grown in fumigated soil; follow the microbial recolonization of the soil and rhizosphere of the seedlings; and determine the resultant mycorrhizal condition of the roots. This paper reports the microbial aspects of the study.

### Materials and Methods

Four areas, 0.1 ha each, were established in a forest nursery located in the Coastal Plain of North Carolina. Methyl bromide fumigant (Nemaster: 68.6 percent methyl bromide, 1.4 percent chloropicrin, and 30 percent inert hydro-carbons) was applied at rates of 0, 170, 340, and 680 kg/ha to a depth of 15 cm with a tractor-mounted soil-injection apparatus. Each fumigated area was sealed for 3 days with a plastic cover. The day after the covers were removed, loblolly pine (*Pinus taeda* L.) seed was sown; and the day after that, a longleaf pine (*P. palustris* Mill.) needle mulch collected the current year was applied to all seedbeds. Four plots, 1.2 × 6 m, were established on each treatment area.

Soil samples were collected several hours before fumigation, several hours after removal of the plastic covers, and at varying intervals thereafter. The rhizosphere was sampled about 2 weeks after seed germination and on eight other occasions. Soil samples were collected in the field with a sterile 2-cm-diameter sampler. Three cores from random points along the length of each plot were placed in a sterile plastic bag and thoroughly shaken to homogenize the sample. Samples were taken from various depths in each plot. All samples were processed within 8 hours after they were taken.

The rhizosphere was sampled by the technique discussed by Rovira (1965). A large block of soil and roots was loosened and the individual trees removed. The soil loosely adhering to the seedling roots was removed by gentle shaking, and about 1 g of the remaining root material was placed directly into 99 ml of sterile water. For the parallel nonrhizosphere samples, three soil cores within 30 cm of the point of rhizospheric sampling were taken at the same depth as root penetration and composited.

Fungi, bacteria, and actinomycetes were enumerated and isolated by the dilution-plate technique. Approximately 1 g of soil was shaken in 99 ml of sterile distilled water on a rotary shaker for 30 minutes. The dilutions were designed to give 15 to 30 fungal colonies or 30 to 100 bacterial and actinomycete colonies per plate. Dextrose-peptone-yeast extract medium with various bacterial and fungal inhibitors (Papavizas and Davey 1959) was used for the isolation of the fungi, and egg albumen medium (Waksman 1922) for the enumeration of bacteria and actinomycetes. For each sample, five replicate plates for fungi and five for bacteria and actinomycetes were prepared, 1 ml of the appropriate dilution and 15 ml of medium at 47° C being added to each plate in a filtered-air transfer room. The bacterial plates were incubated in the dark at 25° C for 10 to 12 days. The fungal plates were incubated in the light at room temperature (23–28° C) for 7 to 8 days unless fast-growing colonies threatened to overgrow the plates; in such case the plates were counted after 3 to 4 days and again after 7 to 8 days.

All calculations were based on the oven-dry weight (70° C) of soil in the initial dilution flasks. For rhizosphere samples the rhizosphere soil was weighed separately from the roots and calculations were based on the soil alone.

Fungal symbionts from the mycorrhizal roots were isolated by the method of Zak and Marx (1964). The optimum time for surface sterilization of root material in 100 ppm $HgCl_2$ solution was found to be between 75 and 120 seconds. Longer exposure usually rendered the material completely sterile. About 50 replicate root segments of each treatment were placed individually on Hagem's agar (Hacskaylo *et al.* 1965) slants. The slants were incubated in the light for 30 to 40 days at 20 to 25° C and periodically examined for the presence of clamp-bearing hyphae. Other fungi which grew on the slants were also counted, and representatives of each type were subcultured for future identification.

To characterize mycorrhizal infection, periodic collections of the roots were fixed and stored in weak formaldehyde-acetic acid-ethanol solution (Johansen 1940) until embedding. Roots were dehydrated in Johansen's tertiary butyl alcohol (TBA) half-step series of solutions. Segments were embedded in hard Tissuemat (Sass 1961), sectioned 10$\mu$ thick, cut, and stained with the fast green-safranin combination of Johansen (1940).

### Soil Fungi

The 0 and 170 kg treatments and the 340 and 680 kg treatments generally formed two discrete groups and only representative results will be presented here. Fumigation almost completely eliminated the native soil fungi (Table 1). The addition of the mulch caused an immediate increase in the total numbers of fungi in the surface soil, although the mulch itself was excluded from all samples. As a result, the effects of fumigation were difficult to detect in the surface soil 10 days after the mulch application. However, the deeper samples clearly showed the long-lasting, drastic effects of soil fumigation on the saprophytic fungal population. The data for the 340 and 680 kg treatments and the 7.5–10 and 12.5–15 cm depths showed very similar recolonization patterns. In all of these cases the fungal populations were reduced to such low levels that they did not recover during the experimental period; at soil depths of 12.5 to 15 cm, fungal populations of soil fumigated at 340 and 680 kg/ha 7 months previously were less than 10 percent that of the control.

The seasonal population pattern was one of gradual decrease in numbers throughout the summer with a large increase in the fall (Table 1). This pattern closely followed changes in the soil moisture content, which was quite low in the late summer and increased in the late autumn. The bacterial populations, but not the actinomycetes, also followed this pattern as will be shown below (cf. Table 4).

The principal recolonizing fungi in the fumigated surface soil were *Trichoderma* spp. and *Penicillium piscarium* (Table 1). These fungi also became quite common in the control areas, but their lower occurrence there indicates that the indigenous population of competing organisms prevented extensive development.

The dominance of *Trichoderma* and *P. piscarium* was maintained throughout the sampling period although their numbers gradually decreased, following the total population pattern. The dominance of *Trichoderma* in fumigated soils has

*TABLE 1. Total number of fungi and percentages of* Trichoderma *spp. and* Penicillium piscarium *colonizing two depths of fumigated and nonfumigated soil.*

| Days after fumigant application[1] | Total fungi at depth (cm) of— | | *Trichoderma* spp. at depth (cm) of— | | *P. piscarium* at depth (cm) of— | |
|---|---|---|---|---|---|---|
| | 0–2.5 | 12.5–15 | 0–2.5 | 12.5–15 | 0–2.5 | 12.5–15 |
| | *$10^3$/g* | | *Percent* | | *Percent* | |
| | | | *Nonfumigated soil* | | | |
| 0[2] | 100 | 83 | 1 | 7 | <1 | <1 |
| 3[3] | 84 | 126 | 5 | 3 | 2 | <1 |
| 6 | 135 | 119 | 23 | 5 | 6 | 1 |
| 15 | 116 | 137 | 18 | 1 | 12 | 2 |
| 28 | 140 | 118 | 18 | 6 | 17 | 1 |
| 75 | 87 | 68 | 15 | 5 | 23 | 1 |
| 116 | 69 | 32 | 6 | 6 | 23 | 2 |
| 214 | 125 | 71 | 2 | 5 | 28 | 1 |
| | | | *340 kg fumigant/ha* | | | |
| 0[2] | 106 | 171 | 3 | 2 | <1 | <1 |
| 3[3] | <1 | 3 | 0 | 7 | <1 | 0 |
| 6 | 4 | <1 | 36 | 4 | 30 | 0 |
| 15 | 153 | 9 | 27 | 54 | 38 | 3 |
| 28 | 119 | 9 | 29 | 13 | 46 | 8 |
| 75 | 71 | 15 | 36 | 28 | 45 | 0 |
| 116 | 40 | 8 | 22 | 16 | 44 | 0 |
| 214 | 95 | 6 | 12 | 34 | 48 | 0 |

[1] Mulch applied on day five.

[2] Immediately before fumigant application.

[3] Immediately after removal of plastic covers.

been almost universally observed, but this is apparently the first report of *P. piscarium* as a dominant recolonizer of fumigated soil.

Although the subsurface layers of fumigated soil contained *Trichoderma* and *P. piscarium*, their numbers never approached those in the surface layer. Factors that may have contributed to this low population are undetected competition, the lack of available carbohydrates, or fungistasis. This numerically low population level in the subsurface soil suggests that conidia in the surface layer apparently failed to move downward even though the soil was coarse-textured and large amounts of water were received through rainfall and irrigation. Hepple (1960) concluded that only on rare occasions would water move significant numbers of spores through the profile.

Excluding yeasts, 61 species of fungi were isolated and identified. The major species are listed in Table 2. One of the principal survivors in the subsurface soil subjected to the 170 kg/ha fumigation rate was *Aureobasidium* (*Pullularia*). It was frequently found in the control soil but was almost completely eliminated at the high levels of fumigation. Domsch (1964) has reported *Pullularia* to be relatively resistant to fumigants.

*Penicillium janthinellum* was one of the most commonly isolated fungi from the control soil, but was almost completely eliminated by fumigation. In the nonfumigated soil it maintained itself at a low but constant level throughout the 8-month sampling period. Hodges (1962) reported that *P. janthinellum* was the most common fungus isolated in a survey of Southern forest nurseries. A second

*TABLE 2. Major species of filamentous fungi isolated from a nursery soil and rhizosphere of loblolly pine. All soil depths and fumigation rates included.*

| Fungi isolated | Occurrence in soil[1] | |
|---|---|---|
| | Non-fumigated | Fumigated |
| *Absidia* sp. | ++ | |
| *Alternaria humicola* Oudemans | + | |
| *Aspergillus fumigatus* Fresenius | +++ | ++ |
| *A. niger* Van Tieghem | +++ | ++ |
| *A. sydowi* (Bain. and Sart.) Thom and Church | +++ | ++ |
| *A. versicolor* (Vuill.) Tiraboschi | ++ | |
| *A. fumigatus* var. *ellipticus* Raper and Fennell | +++ | + |
| *Aureobasidium pullulans* Cooke | ++++ | +++ |
| *Cephalosporium* spp. | +++ | ++ |
| *Chaetomium aureum* Chivers | + | |
| *C. gangligerum* Ames | + | |
| *C. cochlioides* Palliser | ++++ | ++ |
| *C. globosum* Kunze | + | |
| *C. murorum* Corda | + | |
| *Cladosporium herbarium* (Persoon) Link | +++ | ++ |
| *Cladosporium* spp. | ++ | |
| *Coniothyrium fuckelii* Saccardo | +++ | + |
| *Cunninghamella* sp. | ++ | |
| *Diplosporium* spp. | +++ | + |
| *Fusarium* spp. | ++ | + |
| *Gliocladium catenulatum* Gilman and Abbott | ++++ | ++ |
| *Graphium* sp. | ++ | |
| *Leptographium* sp. | + | |
| *Lophotrichus brevirostratus* Brooks | + | |
| *Monocillium* spp. | ++ | |
| *Mucor* spp. | ++ | |
| *Myrothecium verrucaria* (Albertini and Schweinitz) Ditmar | ++ | |
| *Paecilomyces varioti* Bainier | ++ | ++ |
| *Penicillium piscarium* Westling | ++++ | ++++ |
| *P. janthinellum* Biourge | ++++ | + |
| *P. waksmani* Zaleski | +++ | |
| *P. verruculosum* Peyronel | + | |
| *Pestalotia* sp. | + | |
| *Phoma* spp. | +++ | ++ |
| *Rhizopus arrhizus* Fischer | ++ | |
| *Sclerotium bataticola* Taub. | + | |
| *Scopularopsis brevicaulis* Bainer | + | |
| *Stemphyllium* sp. | + | |
| *Trichoderma* spp. | ++++ | ++++ |
| *Volutella* sp. | ++ | |
| *Zygorhynchus moelleri* Vuillemin | ++ | |

[1] Rare +; occasional ++; frequent +++; very abundant ++++.

common fungus that was eliminated by fumigation was *Penicillium waksmani*, demonstrating the potential of fumigation in eliminating specific organisms.

Other common fungi in the nonfumigated soil included *Cephalosporium* spp., *Chaetomium cochlioides*, *Coniothyrium fuckelii*, *Gliocladium catenulatum*, and *Phoma* spp.

Samples of the longleaf pine needle mulch were taken soon after application to the seedbeds to determine the influence of the mulch on the soil microflora. Samples from two sources were studied; one from needles that were collected the current year and subjected to a very weak fumigation about one week before application and one from nonfumigated needles which had been collected the previous year and stored outdoors.

Although the number of species isolated was small, qualitative differences between the two mulch types are clear (Table 3). The fresh mulch microflora was dominated by *Trichoderma* and *P. piscarium* whereas the older mulch was

*TABLE 3. Frequency on dilution plates of the principal fungi isolated from two types of longleaf pine needle mulch.* (*In percent*)

| Fungal species or group | Mulch type | |
|---|---|---|
| | Fresh | One-year-old |
| *Aspergillus fumigatus* | 1 | 1 |
| *A. niger* | 1 | 0 |
| *A. sydowi* | 0 | 1 |
| *Aureobasidium pullulans* | 3 | 0 |
| *Paecilomyces varioti* | 2 | 11 |
| *Penicillium piscarium* | 33 | 1 |
| *Trichoderma* spp. | 20 | 1 |
| Yeasts | 2 | 4 |
| Dark species | 1 | 54 |

dominated by dark species. The fresh mulch would then provide a heavy inoculum of two fast-growing, heavily sporulating fungi which would readily colonize fumigated soil.

To determine if these two microbiologically different mulches would have different effects on fumigated soil, the old mulch was applied to an additional nursery bed fumigated at the 680 kg rate. Samples taken after 140 days indicated significant and long-lasting effects of the mulch type on the microflora of the fumigated soil (Fig. 1). The total number of fungi was greater in the soil with fresh mulch at all three depths. The fresh mulch application also resulted in high populations of *Trichoderma* and *P. piscarium* in the surface soil. The differences in the numbers of these two fungi approximately account for the higher total numbers in the soil with fresh mulch. The moderately high numbers of these fungi in the soil treated with the old mulch indicates that, even though these fungi were not dominant originally in either the soil (see Table 1) or mulch, their rapid growth rate and the reduced competition following fumigation allowed them to establish themselves as dominants.

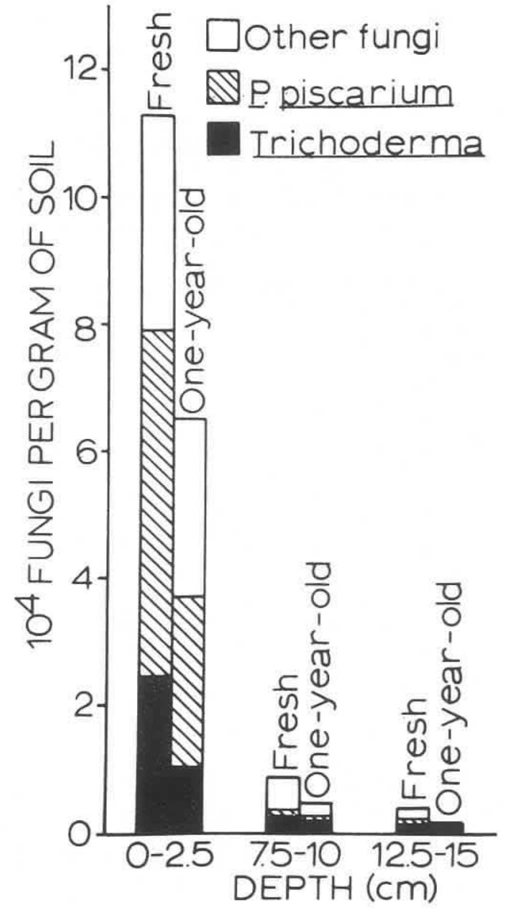

FIGURE 1. *Effect of fresh and 1-year-old longleaf pine needle mulches on fungal populations 140 days after application of 680 kg/ha of methyl bromide fumigant.*

### *Soil Bacteria and Actinomycetes*

The total bacterial populations behaved quite differently than the fungi (Table 4). At all depths and throughout the sampling period bacterial populations in heavily fumigated soils were consistently well above those of the control.

An interesting feature was the immediate and tremendous increase in bacterial numbers in the 680 kg/ha treatment. This response was directly related to depth. Since these samples were taken within 2 to 3 hours after removal of the covers, the population

*TABLE 4. Numbers of bacteria at three depths in nonfumigated and fumigated soil. (In $10^6$ bacteria per gram of soil)*

| Days after fumigant application | Nonfumigated soil; depth (cm)— | | | 340 kg fumigant/ha; soil depth (cm)— | | | 680 kg fumigant/ha; soil depth (cm)— | | |
|---|---|---|---|---|---|---|---|---|---|
| | 0–2.5 | 7.5–10 | 12.5–15 | 0–2.5 | 7.5–10 | 12.5–15 | 0–2.5 | 7.5–10 | 12.5–15 |
| 0[1] | 14 | 13 | 11 | 19 | 23 | 16 | 13 | 14 | 14 |
| 3[2] | 10 | 13 | 11 | 18 | 32 | 29 | 56 | 108 | 197 |
| 6 | 14 | 13 | 12 | 34 | 24 | 23 | 58 | 53 | 27 |
| 15 | 12 | 10 | 10 | 82 | 54 | 30 | 42 | 31 | 44 |
| 28 | 12 | 9 | 9 | 42 | 23 | 24 | 37 | 31 | 22 |
| 75 | 5 | 3 | 3 | 18 | 14 | 11 | 26 | 17 | 23 |
| 116 | 1 | 1 | 2 | 5 | 6 | 3 | 4 | 7 | 6 |
| 201 | 7 | 10 | 12 | 14 | 18 | 16 | 16 | 22 | 23 |

[1] Immediately before fumigant application.

[2] Immediately after removal of plastic covers.

buildup must have begun before the covers were removed. The large population was unstable and 3 days after the covers were removed the numbers in the deeper layers of the 680-kg plots fell abruptly. For the remainder of the sampling period the 340- and 680-kg treatments were similar.

The tremendous initial rise in bacterial numbers can probably be attributed to the growth of survivors (e.g., spore formers) on nutrients liberated from killed microorganisms. The temperature and moisture conditions beneath the clear plastic covers would help provide near optimum conditions for growth. Although no qualitative determinations were made on the bacterial populations either before or following fumigation, it might be hypothesized that facultatively anaerobic *Bacillus* species comprised the major portion of the extremely large populations seen immediately following cover removal. Possibly the fumigant created conditions that allowed these bacteria to grow anaerobically or microaerophilically beneath the covers. When the covers were removed, aerobic conditions would be restored and spore formation initiated.[1] The rapid decrease in numbers indicated by the data (Table 4) could be due to the low germination rate of spores on conventional media (Foerster and Foster 1966).

The striking change in the bacterial population may help explain inconsistencies in previous observations. McKeen (1954) stated that the surviving bacteria were largely spore-formers, whereas Martin (1963) observed that *Pseudomonas* spp. often constituted the majority of the colonies developing on his plates. Differences in isolation media or time of sampling might result in almost totally different conclusions as to the species composition.

In contrast to the fungi and bacteria, numbers of actinomycetes underwent a small depression with all three fumigation treatments at all depths, recovered fairly quickly, and then rose above the control (Table 5). As elsewhere (Alexander 1961), the actinomycetes were little affected by soil depth nor were they as sensitive to summer drying of the soil as the fungi or bacteria.

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[1] Jurgensen, M. F. Personal communication, 1966.

*TABLE 5. Numbers of actinomycetes at two depths in fumigated and nonfumigated soil.*
*(In $10^5$ actinomycetes per gram of soil)*

| Days after fumigant application | Nonfumigated soil; depth (cm)— | | 340 kg fumigant/ha; soil depth (cm)— | |
|---|---|---|---|---|
| | 0–2.5 | 12.5–15 | 0–2.5 | 12.5–15 |
| 0[1] | 19 | 17 | 15 | 18 |
| 3[2] | 17 | 12 | 1 | 7 |
| 6 | 15 | 19 | 5 | 12 |
| 15 | 16 | 11 | 31 | 33 |
| 28 | 11 | 15 | 42 | 45 |
| 75 | 7 | 9 | 33 | 39 |
| 116 | 4 | 7 | 34 | 23 |
| 201 | 1 | 2 | 4 | 4 |

[1] Immediately before fumigant application.
[2] Immediately after removal of plastic covers.

### Root Zone Organisms

Since treatment effects on the rhizosphere fungi were not apparent, the data for the four treatments were pooled (Table 6). The soil population experienced a gradual seasonal decrease in numbers whereas the rhizosphere populations fluctuated, with a particularly notable rise in the late summer. Such population behavior would be expected if the fungi in the root region were strongly influenced by the tree root system.

The most common fungi isolated from rhizosphere and soil were the same, *Trichoderma* and *Penicillium piscarium*. Combined, they composed about one-half to two-thirds of the fungi on the dilution plates. Apparently, the tree roots and fumigation affected the soil microflora somewhat similarly in that each allowed the rapid development of *Trichoderma* and *P. piscarium*. Further analysis of the species composition of the rhizosphere did not reveal any major differences from those found in the soil.

The bacteria were generally much more numerous in the rhizosphere than in root-free soil and qualitative differences were obvious. The predominant bacteria in the root zone were gram-negative rods which formed mucoid colonies. This colony type was rarely seen on plates from root-free soil. The actinomycetes did not respond positively to the influence of pine roots and were often more numerous in the root-free soil than in the rhizosphere.

Of the 19 species of fungi isolated from surface-sterilized mycorrhizae, 7 were not isolated from the rhizosphere (Table 7). These were *Botrytis* sp., *Chaetomium aureum*, *C. gangligerum*, *C. trilaterale*, two species of *Cylindrocarpon* and a clamp-bearing basidiomycete. A greater

*TABLE 6. Total number of fungi and percentages of* Trichoderma *spp. and* Penicillium piscarium *in rhizosphere and nonrhizosphere soil.*[1]

| Days from germination | Nonrhizosphere soil | | | Rhizosphere soil | | |
|---|---|---|---|---|---|---|
| | Total fungi | *Trichoderma* | *P. piscarium* | Total fungi | *Trichoderma* | *P. piscarium* |
| | *$10^3/g$* | *Percent* | *Percent* | *$10^3/g$* | *Percent* | *Percent* |
| 12 | 118 | 26 | 28 | 264 | 30 | 22 |
| 20 | 81 | 32 | 23 | 178 | 36 | 24 |
| 29 | 78 | 25 | 22 | 333 | 44 | 28 |
| 42 | 90 | 30 | 26 | 258 | 37 | 21 |
| 53 | 70 | 23 | 21 | 264 | 31 | 31 |
| 68 | 60 | 25 | 16 | 244 | 23 | 22 |
| 90 | 38 | 10 | 10 | 122 | 19 | 16 |
| 119 | 75 | 15 | 24 | 338 | 7 | 49 |
| 160 | 46 | 12 | 17 | 329 | 4 | 47 |

[1] Figures are the means of all four treatments.

*TABLE 7. Isolation frequency for fungi from surface-sterilized loblolly pine mycorrhizae. (In percent)*

| Fungi isolated | Nonfumigated soil; mycorrhizal type— | | | | Fumigated soil | |
|---|---|---|---|---|---|---|
| | Normal | Dark | Coralloid | All | 340 kg/ha | 680 kg/ha |
| *Aspergillus niger* | 0 | 0 | 0 | 0 | 0 | 1.0 |
| *Botrytis* sp. | 0 | 0 | 0 | 0 | 0 | 19.0 |
| *Cephalosporium* sp. | 0 | 0 | 0 | 0 | 0.4 | 1.0 |
| *Chaetomium aureum* | 4.3 | 4.3 | 3.3 | 3.5 | 2.8 | 0 |
| *C. cochlioides* | 0 | 0 | 0 | 0.3 | 0 | 0 |
| *C. gangligerum* | 10.0 | 7.1 | 4.9 | 5.9 | 0 | 0 |
| *C. trilaterale* Chivers | 7.3 | 7.1 | 1.6 | 6.8 | 16.5 | 0 |
| *Cladosporium* sp. | 0 | 1.4 | 0 | 0.3 | 0 | 0 |
| *Coniothyrium fuckelii* | 1.4 | 0 | 0 | 0.5 | 0 | 0 |
| *Cylindrocarpon* sp. 1 | 0 | 0 | 0 | 1.4 | 0 | 0 |
| *Cylindrocarpon* sp. 2 | 1.4 | 0 | 0 | 0.5 | 0 | 0 |
| *Fusarium* spp. | 7.9 | 2.9 | 0 | 4.3 | 1.4 | 0 |
| *Gliocladium catenulatum* | 1.4 | 0 | 0 | 0.8 | 0 | 0 |
| *Penicillium piscarium* | 5.0 | 0 | 0 | 1.9 | 7.5 | 11.0 |
| *P. janthinellum* | 5.0 | 0 | 0 | 1.9 | 0.9 | 0 |
| *P. verruculosum* | 0.7 | 4.3 | 1.6 | 1.4 | 1.8 | 0 |
| *Phoma* sp. | 0 | 24.3 | 3.3 | 7.0 | 0.9 | 0 |
| *Trichoderma* spp. | 2.2 | 4.3 | 1.6 | 1.9 | 2.8 | 6.0 |
| Clamp-bearing | 13.0 | 0 | 0 | 5.1 | 5.7 | 2.0 |
| Sterile, hyaline | 5.8 | 14.3 | 13.1 | 10.0 | 1.3 | 2.0 |
| Sterile, dark | 7.2 | 1.4 | 0 | 3.0 | 5.6 | 13.0 |
| No. of segments cultured | 139 | 70 | 61 | 370 | 212 | 100 |
| Percent yielding fungi | 91 | 70 | 31 | 64 | 53 | 65 |

number of species was isolated from roots grown in nonfumigated soils than in fumigated soils. Fumigation almost completely eliminated the pathogen-containing genera *Fusarium*, *Phoma*, *Coniothyrium*, and *Cylindrocarpon*. *Chaetomium trilaterale* and *C. gangligerum* were also more commonly isolated from roots from nonfumigated soil. In contrast to this was the exclusive and frequent occurrence (from 19 percent of the root tips) of a *Botrytis* species from roots grown in soil fumigated at the 680 kg/ha rate.

*Botrytis* is a pathogen-containing genus, and its association with roots only in heavily fumigated soil indicates one possible danger of excessive fumigation. Behr (1966) has recently reported increased infestation of plants by *Botrytis cinerea* growing in fumigated soil as compared to plants in untreated soil. He attributed the differences to the elimination of an antagonistic soil microflora. The results of our nursery experiment appear very similar to Behr's results and may have resulted from a similar cause.

*Trichoderma* and *P. piscarium* occurred more frequently on roots from fumigated than from nonfumigated soil. However, they were never the major organisms growing from the roots, indicating they were not as intimately associated with roots as some other fungi despite their extremely high numbers in the rhizosphere. The relatively low recovery of these two species would indicate that most rhizosphere microorganisms were successfully eliminated from the roots by the surface sterilization and washing technique.

### Mycorrhizae

The results of the surface-sterilization study showed the presence of clamp-bearing basidiomycetes in roots from fumigated as well as nonfumigated soil (Table 7). Presumably most of these were mycorrhizal fungi.

The mycorrhizal type found most commonly in fumigated soil was characterized by extremely long and irregularly branched secondary roots. A similar type, but with more regular dichotomous branching and shorter tips, was common in the nonfumigated soil. The external morphology of the mycorrhizae found in the control soil was more diverse and included a coralloid type, indicating a variety of fungal symbionts.

Associated with each type of mycorrhiza from the control soil was a characteristic spectrum of nonmycorrhizal fungi. The principal fungi isolated from the normal type (that type most frequently encountered) were *Chaetomium gangligerum*, *C. trilaterale*, *Fusarium* spp., a clamp-bearing basidiomycete, and two sterile, dark species. The most common fungus isolated from the dark mycorrhizae was *Phoma* sp. 1 (24 percent). Also frequently isolated was *C. gangligerum*, *C. trilaterale*, and a sterile hyaline species. A much lower percentage of fungi grew from the coralloid tips; a sterile, hyaline species was the most frequently isolated. This fungus may be a mycobiont since it was recovered at a high rate from this mycorrhizal type but only rarely from the other types. Neither the dark nor coralloid roots yielded any clamp-bearing hyphae whereas clamp-bearing hyphae grew out of 13 percent of the normal mycorrhizal root tips.

From a large number of histological observations it was determined that fumigation retarded mycorrhiza formation. In the untreated soil many of the roots had a well-developed Hartig net 20 days after germination. The delay in infection of the seedlings in fumigated soil was directly related to the fumigation rate; in the 170 kg treatment it was 1 to 2 weeks, in the 340 kg treatment it was 2 to 3 weeks, and in the 680 kg treatment it was greater than 3 weeks. This delay was similar to that reported by Laiho and Mikola (1964) in Finland.

The most common mycorrhizal type in the nonfumigated areas was found to either lack a mantle or have one that was only weakly developed (Fig. 2A). The Hartig net was well developed and composed of large hyphal cells usually a single layer thick (5 to 10 $\mu$). In contrast, the mycorrhizae from the fumigated areas had a thin but consistently present mantle and a well developed Hartig net consisting of small closely packed fungal cells (Fig. 2B). The differences in mantles and Hartig net cell sizes were not absolute but were fairly consistent.

All of the mycorrhizae examined were of the ectotrophic type whereas Mikola (1965) and Laiho (1965) reported that the ectendotrophic type was most common in northern nurseries. Of the numerous nurseries Laiho sampled in the United States, only the single nursery in Georgia lacked this type. His results combined with those from this study suggest that the ectendotrophic type may be less common in southern than in northern nurseries.

### Conclusions

The recolonizing ability of *Trichoderma* in fumigated soils has been noted by many investigators, although its significance still remains unknown. The antagonism of *Trichoderma* to other fungi may be related to the greater growth of seedlings in fumigated soil. A direct affect may also be involved. Lindsey and Baker (1967) have shown that tomatoes in monoxenic culture with *Trichoderma* were significantly taller than germ-free or *Chaetomium*-infested plants. The nursery inoculation experiment conducted by Ingestad and Nilsson (1964) with *Trichoderma* and the resultant growth stimulation of spruce and pine seedlings provide

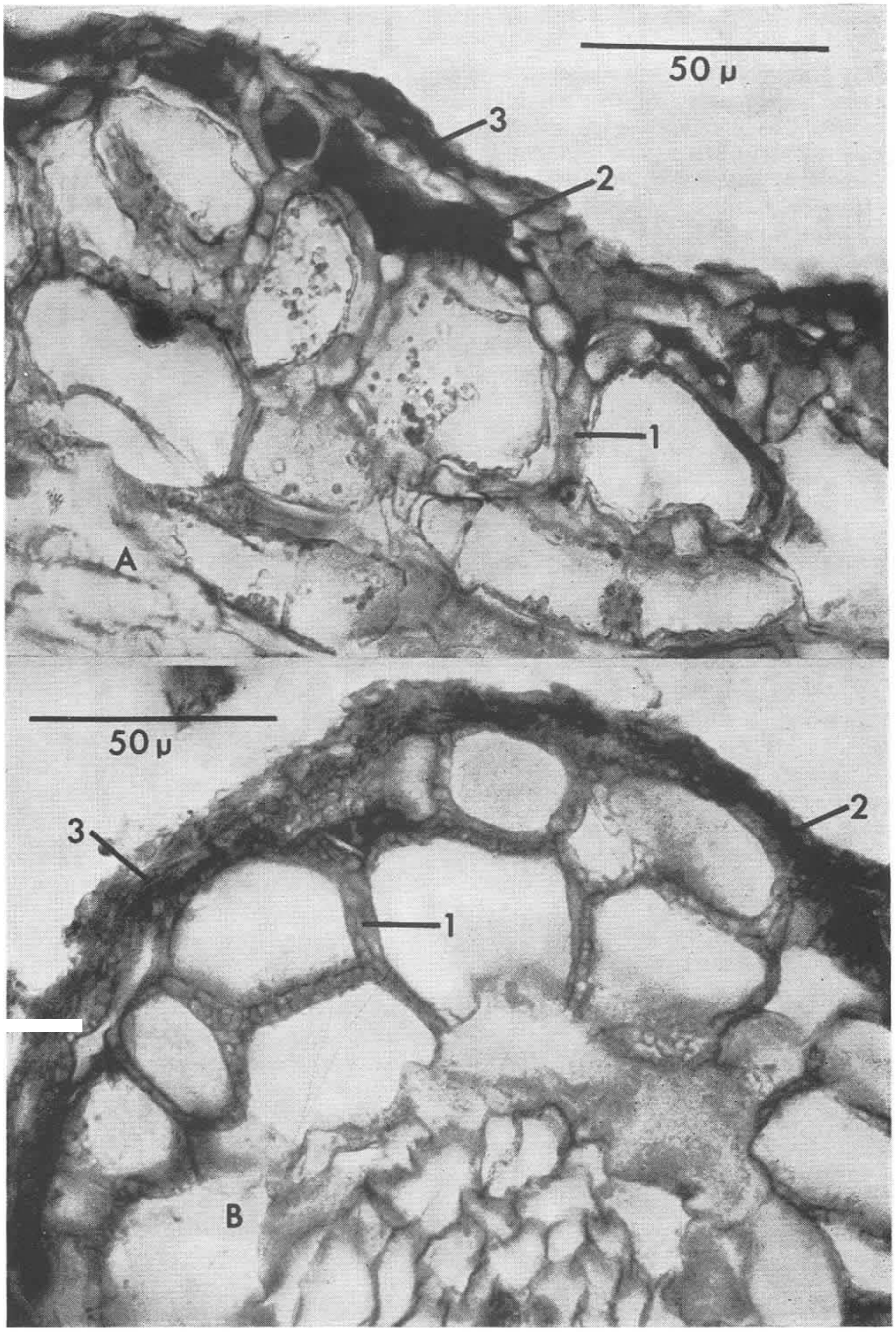

FIGURE 2. *Tangential section of a loblolly pine mycorrhiza from nonfumigated soil (A), and cross-section of a mycorrhiza from fumigated soil (B). Note Hartig net (1), tannin-filled epidermal cells (2), and mantle (3).*

evidence that the cause may be microbial. Thus the parallel development of *Trichoderma* populations following fumigation and the growth response of loblolly pine grown in the fumigated soil may or may not be related. The elimination of potentially pathogenic organisms such as *Fusarium*, *Cylindrocarpon*, *Phoma*, and *Coniothyrium* from the roots may also be important. However, their elimination may be related to stimulation of an antagonistic microflora.

Application of a nonsterilized mulch can strongly influence the microbial recolonization of soil. Thus, under different cultural practices the populations might have developed quite differently with a large amount of variation as Martin (1950) experienced in greenhouse studies. The application of a uniform source of inoculum probably provides a better basis for the study of soil recolonization than relying on accidental (airborne, etc.) inoculation which might allow nonsoil organisms to become temporarily established due to the lack of competing organisms. Similarly, it may prevent the establishment of pathogenic organisms such as occurred in those cases listed by Vaartaja (1964). An example of a potential adverse effect of fumigation was the high incidence of *Botrytis* on roots from the most highly fumigated area (Table 7). The prevention of reinfestation by pathogens in fumigated soil is nearly impossible due to the numerous modes of dispersion of propagules (Vaartaja 1967). However, the application of mulch with an active saprophytic microflora which can fill the biological void in fumigated soils may be of as great a significance in disease control as the initial elimination of pathogens.

The mulch probably also serves as a major source of the inoculum of mycorrhizal fungi. The use of a mulch from forest stands may introduce the species of symbiotic fungi that would be well adapted to the environmental conditions encountered following seedling outplanting. A proper mulch application would at least eliminate the possibility of the production of nonmycorrhizal seedlings which Wright (1957) found to have a very poor outplanting survival rate.

Since fumigation did not alter the nutrient status of the soil but seedling growth was greatest in the fumigated soil,[2] it is concluded that the superior growth is due to a microbial cause. This may occur through the elimination of inconspicuous but detrimental root parasites or changes in the mycorrhizal fungi.

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